

## Appendix A

### Example XX. Quantitative expression analysis of clones in various cells and tissues

The quantitative expression of various NOV genes was assessed using microtiter plates containing RNA samples from a variety of normal and pathology-derived cells, cell lines and tissues using real time quantitative PCR (RTQ-PCR) performed on an Applied Biosystems (Foster City, CA) ABI PRISM® 7700 or an ABI PRISM® 7900 HT Sequence Detection System.

RNA integrity of all samples was determined by visual assessment of agarose gel electropherograms using 28S and 18S ribosomal RNA staining intensity ratio as a guide (2:1 to 2.5:1 28s:18s) and the absence of low molecular weight RNAs (degradation products). Control samples to detect genomic DNA contamination included RTQ-PCR reactions run in the absence of reverse transcriptase using probe and primer sets designed to amplify across the span of a single exon.

RNA samples were normalized in reference to nucleic acids encoding constitutively expressed genes (i.e.,  $\beta$ -actin and GAPDH). Alternatively, non-normalized RNA samples were converted to single strand cDNA (sscDNA) using Superscript II (Invitrogen Corporation, Carlsbad, CA, Catalog No. 18064-147) and random hexamers according to the manufacturer's instructions. Reactions containing up to 10  $\mu$ g of total RNA in a volume of 20  $\mu$ l or were scaled up to contain 50  $\mu$ g of total RNA in a volume of 100  $\mu$ l and were incubated for 60 minutes at 42°C. sscDNA samples were then normalized in reference to nucleic acids as described above.

Probes and primers were designed according to Applied Biosystems *Primer Express* Software package (version I for Apple Computer's Macintosh Power PC) or a similar algorithm using the target sequence as input. Default reaction condition settings and the following parameters were set before selecting primers: 250 nM primer concentration; 58°-60° C primer melting temperature ( $T_m$ ) range; 59° C primer optimal  $T_m$ ; 2° C maximum primer difference (if probe does not have 5' G, probe  $T_m$  must be 10° C greater than primer  $T_m$ ; and 75 bp to 100 bp amplicon size. The selected probes and primers were synthesized by Synthegen (Houston, TX). Probes were double purified by HPLC to remove uncoupled dye and evaluated by mass spectroscopy to verify coupling of reporter and quencher dyes to the 5' and 3' ends of the probe, respectively. Their final concentrations were: 900 nM forward and reverse primers, and 200nM probe.

Normalized RNA was spotted in individual wells of a 96 or 384-well PCR plate (Applied Biosystems, Foster City, CA). PCR cocktails included a single gene-specific probe and primers set or two multiplexed probe and primers sets. PCR reactions were done using TaqMan® One-Step RT-PCR Master Mix (Applied Biosystems, Catalog No. 4313803) following manufacturer's instructions. Reverse transcription was performed at 48° C for 30 minutes followed by amplification/PCR cycles: 95° C 10 min, then 40 cycles at 95° C for 15 seconds, followed by 60° C for 1 minute. Results were recorded as CT values (cycle at which a given sample crosses a threshold level of fluorescence) and plotted using a log scale, with the difference in RNA concentration between a given sample and the sample with the lowest CT value being represented as 2 to the power of delta CT. The percent relative expression was the reciprocal of the RNA difference multiplied by 100. CT values below 28 indicate high expression, between 28

and 32 indicate moderate expression, between 32 and 35 indicate low expression and above 35 reflect levels of expression that were too low to be measured reliably.

Normalized sscDNA was analyzed by RTQ-PCR using 1X TaqMan® Universal Master mix (Applied Biosystems; catalog No. 4324020), following the manufacturer's instructions. PCR amplification and analysis were done as described above.

#### **Panels 1, 1.1, 1.2, and 1.3D**

Panels 1, 1.1, 1.2 and 1.3D included 2 control wells (genomic DNA control and chemistry control) and 94 wells of cDNA samples from cultured cell lines and primary normal tissues. Cell lines were derived from carcinomas (ca) including: lung, small cell (s cell var), non small cell (non-s or non-sm); breast; melanoma; colon; prostate; glioma (glio), astrocytoma (astro) and neuroblastoma (neuro); squamous cell (squam); ovarian; liver; renal; gastric and pancreatic from the American Type Culture Collection (ATCC, Bethesda, MD). Normal tissues were obtained from individual adults or fetuses and included: adult and fetal skeletal muscle, adult and fetal heart, adult and fetal kidney, adult and fetal liver, adult and fetal lung, brain, spleen, bone marrow, lymph node, pancreas, salivary gland, pituitary gland, adrenal gland, spinal cord, thymus, stomach, small intestine, colon, bladder, trachea, breast, ovary, uterus, placenta, prostate, testis and adipose. The following abbreviations are used in reporting the results: metastasis (met); pleural effusion (pl. eff or pl effusion) and \* indicates established from metastasis.

#### **General\_screening\_panel\_v1.4, v1.5, v1.6 and v1.7**

Panels 1.4, 1.5, 1.6 and 1.7 were as described for Panels 1, 1.1, 1.2 and 1.3D, above except that normal tissue samples were pooled from 2 to 5 different adults or fetuses.

#### **ARDAIS Kidney v1.0**

ARDAIS Kidney v1.0 panel included 2 control wells and 44 test samples of human renal cell carcinoma and in some cases matched adjacent normal tissue (NAT) obtained from Ardais (Lexington, MA). RNA from unmatched renal cell carcinoma and normal tissue with gross histopathological assessment of tumor differentiation grade and stage and clinical state of the patient were also obtained from Ardais.

#### **Panel 3D, 3.1 and 3.2**

Panels 3D, 3.1, and 3.2 included two controls, 92 cDNA samples of cultured human cancer cell lines and 2 samples of human primary cerebellum. Cell lines (ATCC, National Cancer Institute (NCI), German tumor cell bank) were cultured as recommended and were derived from: squamous cell carcinoma of the tongue, melanoma, sarcoma, leukemia, lymphoma, and epidermoid, bladder, pancreas, kidney, breast, prostate, ovary, uterus, cervix, stomach, colon, lung and CNS carcinomas.

**A. CG54620-01: GMG55707 (AMF10)**

Expression of gene CG54620-01 was assessed using the primer-probe sets Ag1109 and Ag7087, described in Tables AA and AB. Results of the RTQ-PCR runs are shown in Tables AC, AD, AE, AF and AG.

Table I. Probe Name Ag7087

Primers	Sequences	Length	Start Position
Forward	5'-agctttgtagacaggggactagac-3'	24	500
Probe	TET-5'-atctctcgcacactcctctccggag-3'-TAMRA	25	525
Reverse	5'-ggacacatcaaacaatacttctgt-3'	25	550

Table II. Ardaïs Kidney 1.0

Column A - Rel. Exp.(%) Ag7087, Run 429819095			
Tissue Name	A	Tissue Name	A
Kidney cancer(10A8)	46.0	Kidney cancer(10C6)	0.4
Kidney NAT(10A9)	0.2	Kidney cancer(10C9)	0.0
Kidney cancer(10AA)	10.4	Kidney cancer(10D1)	52.1
Kidney NAT(10AB)	0.3	Kidney cancer(10CA)	0.0
Kidney cancer(10AC)	10.2	Kidney cancer(10D2)	95.3
Kidney NAT(10AD)	0.6	Kidney cancer(10CB)	0.0
Kidney cancer(10B6)	3.5	Kidney cancer(10D4)	20.7
Kidney NAT(10B7)	1.1	Kidney cancer(10CD)	61.1
Kidney cancer(10B8)	0.0	Kidney cancer(10D5)	1.2
Kidney NAT(10B9)	1.3	Kidney cancer(10CE)	21.8
Kidney cancer(10BC)	0.0	Kidney cancer(10D6)	0.0
Kidney NAT(10BD)	2.1	Kidney cancer(10CF)	29.5
Kidney cancer(10BE)	12.5	Kidney cancer(10D8)	12.9
Kidney NAT(10BF)	3.8	Kidney cancer(10CC)	18.7
Kidney cancer(10C2)	0.7	Kidney cancer(10D3)	0.4
Kidney NAT(10C3)	9.0	Kidney NAT(10D9)	7.8
Kidney cancer(10C4)	0.1	Kidney NAT(10DB)	0.0
Kidney NAT(10C5)	0.1	Kidney NAT(10DC)	1.1
Kidney cancer(10B4)	0.0	Kidney NAT(10DD)	0.8
Kidney cancer(10C8)	0.0	Kidney NAT(10DE)	0.0
Kidney cancer(10D0)	100.0	Kidney NAT(10B1)	13.0
Kidney cancer(10C0)	0.0	Kidney NAT(10DA)	0.0

Table III. General\_screening\_panel\_v1.7

Column A - Rel. Exp.(%) Ag7087, Run 318036513			
Tissue Name	A	Tissue Name	A
Adipose	9.3	Gastric ca. (liver met.) NCI-N87	0.0
HUVEC	56.3	Stomach	0.1
Melanoma* Hs688(A).T	0.0	Colon ca. SW-948	0.0
Melanoma* Hs688(B).T	0.0	Colon ca. SW480	0.0
Melanoma (met) SK-MEL-5	0.0	Colon ca. (SW480 met) SW620	0.0
Testis	0.5	Colon ca. HT29	0.0
Prostate ca. (bone met) PC-3	2.1	Colon ca. HCT-116	0.0
Prostate ca. DU145	0.0	Colon cancer tissue	0.2
Prostate pool	0.7	Colon ca. SW1116	0.0
Uterus pool	0.5	Colon ca. Colo-205	0.0
Ovarian ca. OVCAR-3	0.1	Colon ca. SW-48	0.0
Ovarian ca. (ascites) SK-OV-3	0.0	Colon	2.1
Ovarian ca. OVCAR-4	0.7	Small Intestine	1.1
Ovarian ca. OVCAR-5	2.4	Fetal Heart	3.0
Ovarian ca. IGROV-1	0.0	Heart	1.0
Ovarian ca. OVCAR-8	100.0	Lymph Node Pool	1.2
Ovary	1.9	Lymph Node pool 2	8.5
Breast ca. MCF-7	0.0	Fetal Skeletal Muscle	
Breast ca. MDA-MB-231	0.0	Skeletal Muscle pool	0.1
Breast ca. BT 549	0.6	Skeletal Muscle	0.4
Breast ca. T47D	0.0	Spleen	0.3
113452 mammary gland	4.7	Thymus	0.0
Trachea	2.6	CNS cancer (glio/astro) SF-268	27.5
Lung	2.6	CNS cancer (glio/astro) T98G	0.2
Fetal Lung	4.2	CNS cancer (neuro;met) SK-N-AS	0.0
Lung ca. NCI-N417	0.0	CNS cancer (astro) SF-539	4.6
Lung ca. LX-1	0.0	CNS cancer (astro) SNB-75	1.0
Lung ca. NCI-H146	0.0	CNS cancer (glio) SNB-19	3.4
Lung ca. SHP-77	0.0	CNS cancer (glio) SF-295	0.0
Lung ca. NCI-H23	1.1	Brain (Amygdala)	1.8
Lung ca. NCI-H460	0.0	Brain (Cerebellum)	0.5
Lung ca. HOP-62	0.4	Brain (Fetal)	8.3
Lung ca. NCI-H522	2.5	Brain (Hippocampus)	1.4

Lung ca. DMS-114	73.2	Cerebral Cortex pool	1.6
Liver	1.4	Brain (Substantia nigra)	0.7
Fetal Liver	3.7	Brain (Thalamus)	2.9
Kidney pool	1.4	Brain (Whole)	5.7
Fetal Kidney	3.0	Spinal Cord	0.7
Renal ca. 786-0	9.1	Adrenal Gland	1.6
Renal ca. A498	0.0	Pituitary Gland	0.7
Renal ca. ACHN	2.5	Salivary Gland	0.0
Renal ca. UO-31	0.0	Thyroid	4.0
Renal ca. TK-10	0.0	Pancreatic ca. PANC-1	0.0
Bladder	10.1	Pancreas pool	1.3

Table IV. Oncology\_cell\_line\_screening\_panel\_v3.2

Column A - Rel. Exp.(%) Ag7087, Run 323342064			
Tissue Name	A	Tissue Name	A
94905 Daoy Medulloblastoma/Cerebellum	0.0	94954 Ca Ski Cervical epidermoid carcinoma (metastasis)	0.0
94906 TE671 Medulloblastoma/Cerebellum	0.0	94955 ES-2 Ovarian clear cell carcinoma	0.2
94907 D283 Med Medulloblastoma/Cerebellum	0.0	94957 Ramos Stimulated with PMA/ionomycin 6h	0.0
94908 PFSK-1 Primitive Neuroectodermal/Cerebellum	0.0	94958 Ramos Stimulated with PMA/ionomycin 14h	0.0
94909 XF-498 CNS	10.2	94962 MEG-01 Chronic myelogenous leukemia (megokaryoblast )	0.6
94910 SNB-78 CNS/glioma	27.2	94963 Raji Burkitt's lymphoma	0.0
94911 SF-268 CNS/glioblastoma	38.4	94964 Daudi Burkitt's lymphoma	0.0
94912 T98G Glioblastoma	0.0	94965 U266 B-cell plasmacytoma/myeloma	0.0
96776 SK-N-SH Neuroblastoma (metastasis)	3.3	94968 CA46 Burkitt's lymphoma	0.0
94913 SF-295 CNS/glioblastoma	0.6	94970 RL non-Hodgkin's B-cell lymphoma	0.0
132565 NT2 pool	3.2	94972 JM1 pre-B-cell lymphoma/leukemia	0.0
94914 Cerebellum	0.0	94973 Jurkat T cell leukemia	0.3
96777 Cerebellum	0.3	94974 TF-1 Erythroleukemia	0.0
94916 NCI-H292 Mucoepidermoid lung carcinoma	0.0	94975 HUT 78 T-cell lymphoma	0.0

94917 DMS-114 Small cell lung cancer	100.0	94977 U937 Histiocytic lymphoma	0.0
94918 DMS-79 Small cell lung cancer/neuroendocrine	1.0	94980 KU-812 Myelogenous leukemia	0.0
94919 NCI-H146 Small cell lung cancer/neuroendocrine	0.0	769-P- Clear cell renal carcinoma	0.0
94920 NCI-H526 Small cell lung cancer/neuroendocrine	0.0	94983 Caki-2 Clear cell renal carcinoma	0.0
94921 NCI-N417 Small cell lung cancer/neuroendocrine	0.0	94984 SW 839 Clear cell renal carcinoma	0.0
94923 NCI-H82 Small cell lung cancer/neuroendocrine	0.0	94986 G401 Wilms' tumor	0.2
94924 NCI-H157 Squamous cell lung cancer (metastasis)	2.8	126768 293 cells	0.0
94925 NCI-H1155 Large cell lung cancer/neuroendocrine	1.3	94987 Hs766T Pancreatic carcinoma (LN metastasis)	0.0
94926 NCI-H1299 Large cell lung cancer/neuroendocrine	18.9	94988 CAPAN-1 Pancreatic adenocarcinoma (liver metastasis)	0.0
94927 NCI-H727 Lung carcinoid	0.4	94989 SU86.86 Pancreatic carcinoma (liver metastasis)	0.0
94928 NCI-UMC-11 Lung carcinoid	0.0	94990 BxPC-3 Pancreatic adenocarcinoma	0.0
94929 LX-1 Small cell lung cancer	0.0	94991 HPAC Pancreatic adenocarcinoma	0.0
94930 Colo-205 Colon cancer	0.0	94992 MIA PaCa-2 Pancreatic carcinoma	0.0
94931 KM12 Colon cancer	0.0	94993 CFPAC-1 Pancreatic ductal adenocarcinoma	0.0
94932 KM20L2 Colon cancer	0.0	94994 PANC-1 Pancreatic epithelioid ductal carcinoma	0.0
94933 NCI-H716 Colon cancer	0.0	94996 T24 Bladder carcinoma (transitional cell)	0.0
94935 SW-48 Colon adenocarcinoma	0.0	5637- Bladder carcinoma	0.0
94936 SW1116 Colon adenocarcinoma	0.0	94998 HT-1197 Bladder carcinoma	0.0
94937 LS 174T Colon adenocarcinoma	0.0	94999 UM-UC-3 Bladder carcinoma (transitional cell)	1.1
94938 SW-948 Colon adenocarcinoma	0.0	95000 A204 Rhabdomyosarcoma	0.0
94939 SW-480 Colon adenocarcinoma	0.0	95001 HT-1080 Fibrosarcoma	2.8
94940 NCI-SNU-5 Gastric carcinoma	0.0	95002 MG-63 Osteosarcoma (bone)	77.4
112197 KATO III Stomach	0.0	95003 SK-LMS-1 Leiomyosarcoma (vulva)	0.0
94943 NCI-SNU-16 Gastric carcinoma	0.0	95004 SJRH30 Rhabdomyosarcoma (met to bone marrow)	0.0
94944 NCI-SNU-1 Gastric carcinoma	0.0	95005 A431 Epidermoid carcinoma	0.0
94946 RF-1 Gastric adenocarcinoma	0.0	95007 WM266-4 Melanoma	0.0

94947 RF-48 Gastric adenocarcinoma	0.0	112195 DU 145 Prostate	0.0
96778 MKN-45 Gastric carcinoma	0.0	95012 MDA-MB-468 Breast adenocarcinoma	0.0
94949 NCI-N87 Gastric carcinoma	0.0	112196 SSC-4 Tongue	0.0
94951 OVCAR-5 Ovarian carcinoma	0.0	112194 SSC-9 Tongue	0.0
94952 RL95-2 Uterine carcinoma	2.7	112191 SSC-15 Tongue	0.1
94953 HeLaS3 Cervical adenocarcinoma	0.0	95017 CAL 27 Squamous cell carcinoma of tongue	0.0